Antimicrobial susceptibility and plasmid profile of *Neisseria gonorrhoeae* in India (New Delhi)

P Bhalla, K Sethi, B S N Reddy, M D Mathur

**Objectives:** To determine the antibiotic susceptibility and plasmid profile of all *Neisseria gonorrhoeae* strains (PPNG and non-PPNG) isolated from May 1995 to March 1996 in Lok Nayak Hospital, New Delhi, India.

**Methods:** The agar plate dilution method was used to determine the minimum inhibitory concentration of five antimicrobials including norfloxacin and ceftriaxone which are most commonly used for treatment of gonorrhoea in Delhi. Isolates were screened for production of penicillinase by paper acidimetric method and plasmid analysis of PPNG and non-PPNG was carried out by agarose gel electrophoresis.

**Results:** 50 consecutive isolates of *N gonorrhoeae* were studied, 8% among them were found to be PPNG while 28% were highly resistant to tetracycline (TRNG). Reduced susceptibility to norfloxacin (MIC $\geq$ 1 $\mu$g/ml) was observed in 12% of all isolates. All PPNG harboured the 4.4 MDa $\beta$ lactamase plasmid along with the 25.2 MDa tetracycline resistance plasmid. Norfloxacin resistance (MIC $\geq$ 1 $\mu$g/ml) was present in 28.5% of TRNG but only in 5.5% of the other gonococcal isolates.

**Conclusions:** Results of this study clearly demonstrate that antibiotic resistant gonococcal strains of different clones are frequently found in New Delhi. Continued surveillance of susceptibility to currently prescribed antimicrobials and epidemiological studies are essential to prevent treatment failures leading to further spread of resistant strains.

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Keywords: antimicrobial susceptibility; *Neisseria gonorrhoeae*; plasmids; India

**Introduction**

The worldwide prevalence of gonorrhoea and the emergence of antibiotic resistant *Neisseria gonorrhoeae* reinforce the need for epidemiological study of this organism and surveillance of its susceptibility to antibiotics commonly used for therapy. Despite a high prevalence of gonorrhoea and an increasing incidence of penicillinase producing *N gonorrhoeae* (PPNG) in India no standardised monitoring of antimicrobial susceptibility of *N gonorrhoeae* has been adopted so far. During 1994–5 we carried out a preliminary study on antimicrobial susceptibility of gonococcal isolates in Lok Nayak Hospital, New Delhi and found 5.5% of them to be PPNG (unpublished data). Plasmid analysis of eight PPNG isolates in Bombay, India revealed the presence of a 4.4 megadalton (MDa) $\beta$ lactamase plasmid in all the isolates, while three of them also carried the large 24.5 MDa conjugative plasmid.

Plasmid mediated high level resistance to tetracycline was first observed in 1985 in the United States. Since then tetracycline resistant *N gonorrhoeae* (TRNG) strains have been reported from England and central Africa. Although tetracycline resistance in *N gonorrhoeae* has been reported from India, there have been no studies on plasmid mediated high level tetracycline resistance.

Fluoroquinolones are highly effective as oral single dose treatment for gonorrhoea but reduced susceptibility and treatment failures with these agents have been recently reported. In Hong Kong, quinolone resistance in *N gonorrhoeae* (QRNG) increased from 0.5% in 1992 to 10.4% in 1994 and was associated with a rapid decline in prevalence of both PPNG and TRNG.

The present study was undertaken to determine the antibiotic susceptibility of *N gonorrhoeae* isolates, to assess the prevalence of PPNG, and to study the plasmid profile of PPNG and non-PPNG strains.

**Material and methods**

**BACTERIAL ISOLATES**

Fifty consecutive isolates of *N gonorrhoeae* recovered from 48 men with urethritis and two women with endocervicitis, attending the STD clinic of Lok Nayak Hospital, New Delhi, between May 1995 and March 1996 were studied.

For isolation of *N gonorrhoeae*, urethral and endocervical specimens were directly inoculated in the STD clinic on modified Thayer–Martin medium (GC agar base + haemoglobin + growth supplement + VCNT) and chocolate agar (GC agar + haemoglobin + growth supplement). The inoculated culture plates were incubated at 34–36°C in a humid atmosphere containing 3–10% carbon dioxide (candle extinction jar) for 48–72 hours. Isolates were identified as *N gonorrhoeae* on the basis of colony morphology, Gram staining, oxidase test, and carbohydrate degradation tests.

Isolates were maintained by daily subculture on chocolate agar or by storage of a bacterial suspension in trypticase soya broth with 20% glycerol at −20°C.
Table 1  MIC values of five antimicrobial agents for 46 non-PPNG isolates of *N. gonorrhoeae*

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>MIC Range (µg/ml)</th>
<th>No of Isolates</th>
<th>No of Resistant Strains</th>
<th>Type of Antimicrobial Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>≤0.15</td>
<td>31</td>
<td>38</td>
<td>CMRNG-Tet</td>
</tr>
<tr>
<td></td>
<td>0.25–16</td>
<td>16</td>
<td>18</td>
<td>QPN+QRNG</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>≤0.25</td>
<td>24</td>
<td>12</td>
<td>CMRNG-Tet</td>
</tr>
<tr>
<td></td>
<td>0.5–16</td>
<td>2</td>
<td>0</td>
<td>QPN+QRNG</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>≤0.007</td>
<td>2</td>
<td>2</td>
<td>TRNG</td>
</tr>
<tr>
<td></td>
<td>0.015–8</td>
<td>0</td>
<td>0</td>
<td>TRNG</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≤0.007</td>
<td>1</td>
<td>1</td>
<td>TRNG</td>
</tr>
<tr>
<td></td>
<td>0.015–8</td>
<td>0</td>
<td>0</td>
<td>TRNG</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>≤0.25</td>
<td>5</td>
<td>4</td>
<td>PPNG</td>
</tr>
<tr>
<td></td>
<td>0.5–32</td>
<td>0</td>
<td>0</td>
<td>PPNG</td>
</tr>
</tbody>
</table>

CMRNG-Tet = chromosomally mediated resistance in *N. gonorrhoeae* to tetracycline (MIC 2–8 µg/ml); CMRNG Pen = chromosomally mediated resistance in *N. gonorrhoeae* to penicillin (MIC ≥1.2 µg/ml); TRNG = tetracycline resistant *N. gonorrhoeae* (MIC ≥16 µg/ml); PPNG = penicillinase producing *N. gonorrhoeae*; QPN = quinolone resistant *N. gonorrhoeae* (MIC ≥1 µg/ml).

**Antimicrobial Susceptibility Tests**

All *N. gonorrhoeae* isolates were tested for production of penicillinase by the paper agrode method. Each isolate was examined for susceptibility to penicillin (0.15–9.6 µg/ml), tetracycline (0.25–16 µg/ml), chloramphenicol (0.25–32 µg/ml), norfloxacin and ceftriaxone (0.007–1 µg/ml) using the agar plate dilution method for determination of minimum inhibitory concentration (MIC). The bacterial suspension used as inoculum was prepared in Mueller–Hinton broth and consisted of approximately 10⁷ cfu/ml. The medium employed for agar plate dilution tests was chocolate agar.

The WHO *N. gonorrhoeae* reference strains A–E, kindly provided by Dr I Lind, Statens Serum Institut, Copenhagen, were used as controls.

**Plasmid Analysis**

All *N. gonorrhoeae* isolates were subjected to plasmid analysis by agarose gel electrophoresis with ethidium bromide staining. Plasmid DNA was extracted and purified from an overnight culture of one ml of each isolate in Mueller–Hinton agar. The plasmid DNA was digested with restriction enzymes and separated by agarose gel electrophoresis. The molecular weight of each plasmid DNA band was calculated and its molecular weight derived.

**Discussion**

In this study population we found 8% gonococcal isolates to be PPNG, although an earlier study in a different hospital in Delhi found no PPNG among 102 isolates examined. The incidence of PPNG reported from other parts of India varies from 0–13.6% but is reported to be as high as 59% from central Africa. All the PPNG strains in the present study were non-PPNG, which was in accordance with previous studies. This has not been reported in any previous Indian study, although one study observed the presence of PPNG in all eight PPNG examined, accompanied by the 24.5 MDa large conjugative plasmid in three PPNG. In Spain the 4.5 MDa type plasmid has been reported to be present in 92.8% of PPNG strains.
PPNG and the 24.5 MDa conjugative plasmid has also been found more frequently among PPNG than among non-PPNG (85% vs 25%).

Plasmid mediated high level resistance to tetracycline, which was first observed in 1985 in the United States, has recently been reported in 10% of gonococcal isolates in central Africa. The proportion of TRNG strains which were also PPNG varies from less than 1% to 81%. We observed high level tetracycline resistance in 28% of gonococcal isolates and all of them harboured the 25.2 MDa plasmid. Four (28.5%) of them were also PPNG, while four (28.5%) were QRNG (MIC > 1 µg/ml). Two different plasmid profiles were observed among TRNG.

A decrease in susceptibility of gonococcal isolates to the fluoroquinolones has already been reported from Rwanda and Hong Kong. In our study, 12 (24%) gonococcal isolates had an MIC of ≥0.06 µg/ml, while six among these had an MIC of ≥1 µg/ml of norfloxacin. There is little information about the relation between the in vitro MIC of fluoroquinolones against gonococci and clinical efficacy except one study which found that failure with an 800 mg single dose of norfloxacin occurred more often in patients with strains having MIPC ≥0.06 µg/ml. None of the studies mentioned the detection of quinolone resistance more frequently among TRNG or PPNG. However, we found norfloxacin resistance to be more frequent among TRNG strains compared with non-TRNG strains (28.5% vs 5.5%).

The results of our study indicate clearly that plasmid mediated resistance to penicillin and tetracycline is rapidly increasing as is resistance to quinolones which were introduced as first line therapy only around 1990. Currently, ceftriaxone is being preferred as first line therapy but unless continued monitoring of antimicrobial susceptibility of gonococcal strains circulating in a community is carried out, treatment failures may occur and lead to prolonged infectiousness and spread of multi-resistant strains of N. gonorrhoeae.

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